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LEVEL II

THE EXPERIMENTAL PATHOLOGY OF THE LAKE LINDU STRAIN OF SCHISTOSOMA JAPONICUM IN THE CRAB-EATING MACAQUE (Macaca fascicularis) IN INDONESIA

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THE EXPERIMENTAL PATHOLOGY OF THE LAKE LINDU STRAIN OF SCHISTOSOMA JAPONICUM IN THE CRAB-EATING MACAQUE (Macaca fascicularis) IN INDONESIA.

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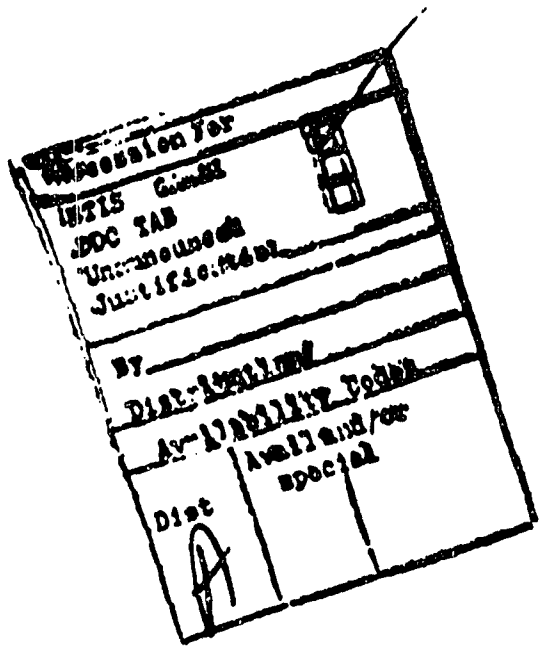
SUMMARY PAGE

THE PROBLEM

The detailed pathology of the Lake Lindu, Central Sulawesi, Indonesia, strain of Schistosoma japonicum has not been described in experimentally infected non-human primates. This study describes the light microscopy lesions caused by experimental infection with this trematode pathogen.

FINDINGS

Two male Macaca fascicularis monkeys were exposed in the laboratory to Schistosoma japonicum cercariae obtained from Onchomelina hupensis lindoensis snails from Lake Lindu, Central Sulawesi (Celebes), Indonesia. The animals were sacrificed at four and six months post infection. Pathologic observations and results of serial stool examination for Schistosoma ova are described.



*The animals used in this study were handled in accordance with the Principles of Laboratory Animal Care established by the Committee on the Guide for Laboratory Animal Resources, National Academy of Science, National Research Council.

INTRODUCTION

The detailed pathology of the Lake Lindu, Sulawesi, Indonesia, strain of Schistosoma japonicum has not been described in experimentally infected nonhuman primates. This study describes the light microscopy lesions caused by the trematode.

Two male Macaca fascicularis monkeys were exposed to Schistosoma japonicum cercariae obtained from Onchomelina snails from Lake Lindu, northern Sulawesi (Celebes), Indonesia. The animals were sacrificed at four to six months post infection and the microscopic lesions and stool samples are described in this paper.

PROCEDURE

Two young adult male Macaca fascicularis monkeys (crab-eating macaque), were exposed to freshly harvested Schistosoma japonicum cercariae from laboratory reared Oncomelina hupensis lindoensis snails. The two animals were anesthetized with Sernylan intramuscularly at a dosage of 3.3 mg per pound and were maintained in an immobile state for a period of approximately 2 hours. The Onchomelina snails were crushed under water and cercariae picked up with a bacteriology loop after they had floated to the surface. The cercariae were counted under a dissecting microscope as they were deposited on the shaved abdomens of the monkeys. The water bearing the cercariae was allowed to dry on the abdomen of the monkey, and he was allowed to recover in his cage. Animal A weighed 4.5 kilograms and was exposed to 2,005 cercariae per-cutaneously on April 30, 1976, while Animal B, weighing 5.5 kilograms was exposed to 501 cercariae per-cutaneously on March 4, 1976.

Twice daily clinical observations of the animal were performed and stool examinations for S. japonicum eggs were made several times weekly.

After 151 days, in Animal A, and 173 days in Animal B, the animals were sacrificed, and a complete gross and microscopic pathology examination was accomplished.

RESULTS

ANIMAL A

Gross Observations

Lesions were identified in the large intestine, liver, mesenteric lymph nodes, and a few foci in the lungs. All other tissues and organs appeared within normal limits.

The large intestine contained segmental areas of scarring and thickening of the intestinal wall, involving the sub-serosa as well as at the mesenteric-intestinal junction, from the cecum to the rectum. All mesenteric lymph nodes were enlarged, firm, and had a rubbery consistency upon cutting. There were adhesions of the omentum to the serosal surface of the large bowel at numerous sites. The mucosa of the large intestine was thickened, rugose, raised, and hemorrhagic in many places. The liver surface revealed

numerous 1mm raised whitish firm nodules of increased density, probably granulomas. A generalized accentuation of the portal areas gave the surface a reticulated appearance.

Microscopic Observations

Microscopically, Animal A demonstrated numerous multiple foci of Schistosoma ova in the lamina propria of the mucosa, the sub-mucosa, muscularis externa, and serosa of the large intestine (Figure 1). Some Schistosoma ova elicited virtually no inflammatory response, while others invoked a significant granulomatous response with Langerhans' giant cells, epithelioid cells, and lymphocytes surrounding the ova while still other ova simply were lying free in a pool of neutrophils and eosinophils in the muscularis of the intestinal wall.

Figure 1. Schistosoma ova in mucosa and sub-mucosa of the large intestine (arrow) of the crab-eating macaque: Experimental infection with Schistosoma [illegible], Lake Lindu, Indonesia, strain. Hematoxylin and Eosin X160.

The liver of Animal A exhibited multiple large granulomas comprised of three to five Schistosoma ova with numerous Langerhans' giant cells all surrounded by a rich fibroblastic response (Figure 2). These granulomas were seen throughout numerous sections of the liver. A significant amount of blood pigment discharged by the flukes had been engulfed by macrophages and the hepatic Kupffer cells. This appeared as intracellular black granules.

Figure 2. Schistosoma japonicum, Lake Lindu strain, experimental infection in the Macaca fascicularis liver. Note the granulomatous reaction containing Schistosoma ova connecting portal triads. Hematoxylin and Eosin X63.

The mesenteric lymph nodes contained a small number of ova surrounded by granulomas. Epithelioid and Langerhans' giant cells were prominent. A few ova elicited virtually no inflammatory response in the lymph nodes.

Sections of myocardium, kidney, skeletal muscle, spleen, and rectum in Animal A were within normal limits.

ANIMAL B

Gross Observations

All internal organs appeared to be within normal limits with the exception of the large intestine, liver, and mesenteric lymph nodes. Multiple foci of scarring were present at the junction of the mesentery and the large intestine throughout its entire length from the cecum to the terminal colon. There was occasional thickening of the gut wall within the transverse, descending, and sigmoid colon, with multiple focal subserosal thickenings of 1 to 2 millimeters in diameter. All mesenteric lymph nodes were enlarged, thickened, firm, and cut with a rubbery consistency. The liver revealed an irregular pattern of scarring on the cut and uncut surfaces.

Microscopic Observations

The granulomatous response of Animal B was essentially the same as that of Animal A in the liver, large intestine, and mesenteric lymph nodes. For this reason the detailed microscopic description of the lesions in Animal B is omitted.

The kidney, bladder, epididymus, testicle, myocardium, skeletal muscle, and spleen of both animals were within normal limits.

Animal A with an exposure of 2,005 cercariae lost 0.4kg (10% weight loss) during the course of its disease, while Animal B with an exposure of 501 cercariae gained 0.72kg(13.3% weight gain) during the disease course.

DISCUSSION

As was expected, the severity of disease in Animal A was greater than in Animal B due to the four-fold cercariae exposure load. The degree and severity of the lesions in Animal A were approximately twice that of Animal B.

The number of eggs counted per gram of stool does not correlate closely with the histopathologic findings. The numbers on Animal A were approximately ten times as high as those of Animal B throughout the patent period of the disease. See Tables I and II.

Table I

Host species:	Macaca fascicularis #A
Age and Sex:	Young adult, male
Weight:	4.5 kilograms
Parasite:	Schistosoma japonicum cercariae
Number of cercariae:	2005
Snail:	Laboratory reared Oncomelania hupensis lindoensis
Methods of Exposure.	By loop per cutaneously
Date of Exposure:	30 April 1976
Date of Necropsy:	1 September 1976
Weight at Necropsy:	4.09 kilograms

Results of Stool Examinations:

Date	Stool Consistence	# eggs per gram stool
11 June 1976	Diarrhea	1218
14 June	Diarrhea	5018
15 June	Diarrhea	3250
16 June	Diarrhea	3582
17 June	Diarrhea	2406
18 June	Diarrhea	3534
21 June	Bloody diarrhea	9746
22 June	Bloody diarrhea	4994
23 June	Bloody diarrhea	3092
24 June	Mushy diarrhea	3740
25 June	Mushy diarrhea	3712
28 June	Mushy diarrhea	4044
29 June	Bloody diarrhea	10420
30 June	Diarrhea	3048
1 July	Bloody diarrhea	6228
2 July	Bloody mushy	3224
6 July	Diarrhea	3204
8 July	Diarrhea	5366
9 July	Bloody diarrhea	6918
12 July	Bloody diarrhea	6838
13 July	Bloody diarrhea	9860
14 July	Diarrhea	3500
15 July	Bloody diarrhea	4798
16 July	Diarrhea	4060
19 July	Bloody diarrhea	4658
20 July	Bloody diarrhea	9662
22 July	Mushy diarrhea	7306
30 July	Mushy diarrhea	5642
6 August	Mushy diarrhea	5876
13 August	Mushy diarrhea	2968
20 August	Mushy diarrhea	2520
27 August	Mushy diarrhea	2554

Table II

Host species:	Macaca fascicularis #8
Age and sex:	Young adult, male
Weight:	5.45 kilogram
Parasite:	Schistosoma japonicum cercariae
Number of cercariae:	501
Snail:	Laboratory reared Oncomelania hupensis lindoensis
Methods of Exposure:	By loop, per-cutaneously
Date of Exposure:	4 March 1976
Date of Necropsy:	27 August 1976
Weight at Necropsy:	6.18 kilogram

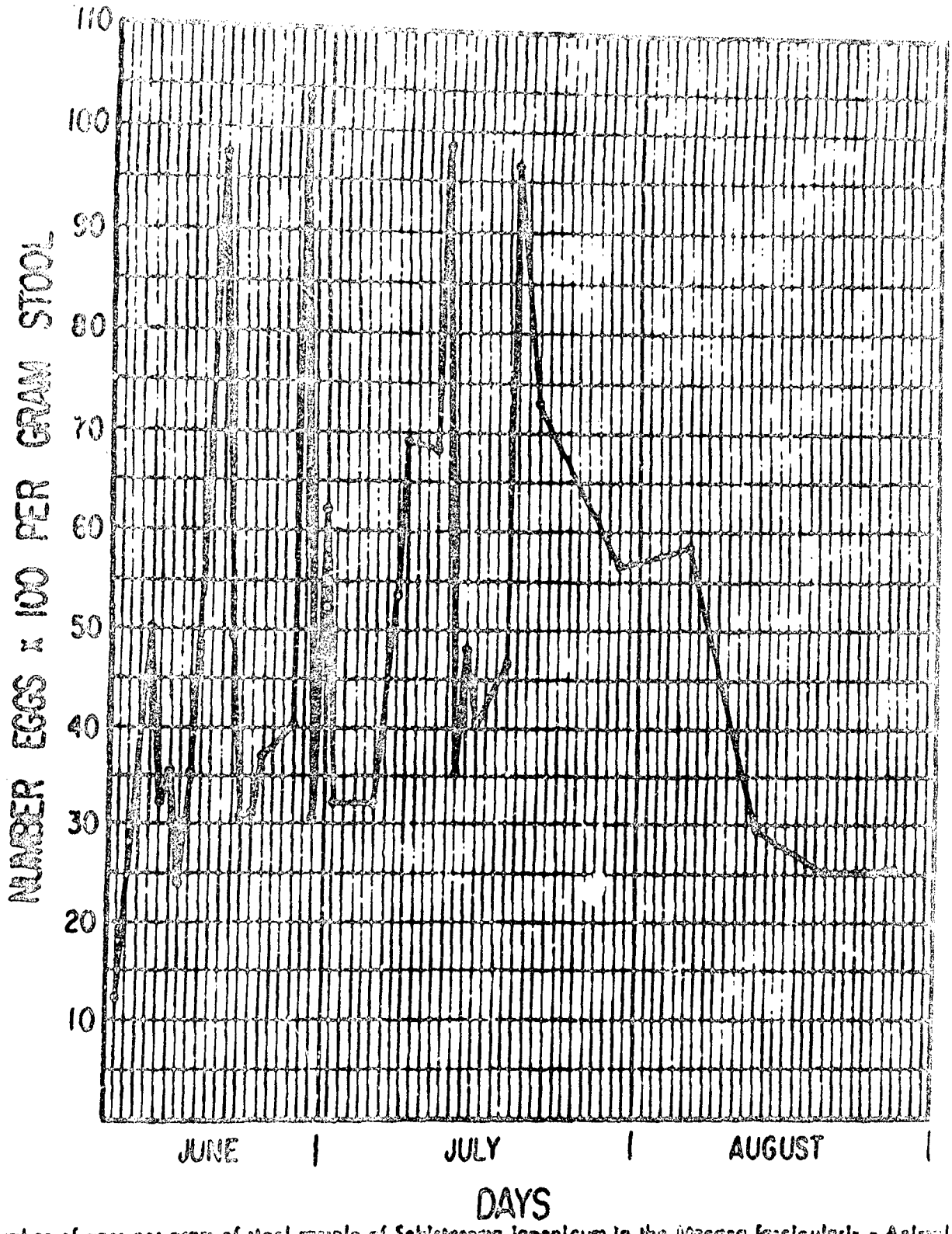
Results of Stool Examinations:

Date	Ova and Parasite and Stool Consistence	# eggs per gram stool
12 Mar 1976	Entamoeba, Balantidium, Hookworm	
19 Mar	Entamoeba, Iodamoeba, Balantidium, Hookworm	
26 Mar	Entamoeba, Hookworm	
15 Apr	Entamoeba, Balantidium, Hookworm	
21 Apr	S. japonicum, Hookworm, Entamoeba	
	Bloody	283
22 Apr	Bloody	541
23 Apr	Bloody mucus	1331
26 Apr	Mushy bloody	515
27 Apr	Mushy bloody	412
28 Apr	Mushy bloody	379
29 Apr	Mushy bloody	200
30 Apr	Mushy	164
3 May	Mushy	314
4 May	Mushy	408
5 May	Mushy bloody	407
6 May	Mushy	522
7 May	Mushy	496
10 May	Mushy	332
11 May	Mushy	1652
12 May	Mushy	632
13 May	Mushy	326
14 May	Mushy	456
17 May	Mushy	242
18 May	Mushy	354
19 May	Mushy	396
20 May	Mushy	426
21 May	Mushy bloody	1022
24 May	Mushy	346
25 May	Mushy formed bloody	324
26 May	Mushy bloody	1566
28 May	Mushy	302
31 May	Mushy bloody	3076
1 June	Mushy bloody	902
2 June	Mushy formed bloody	978
3 June	Formed	296
4 June	Mushy formed	318

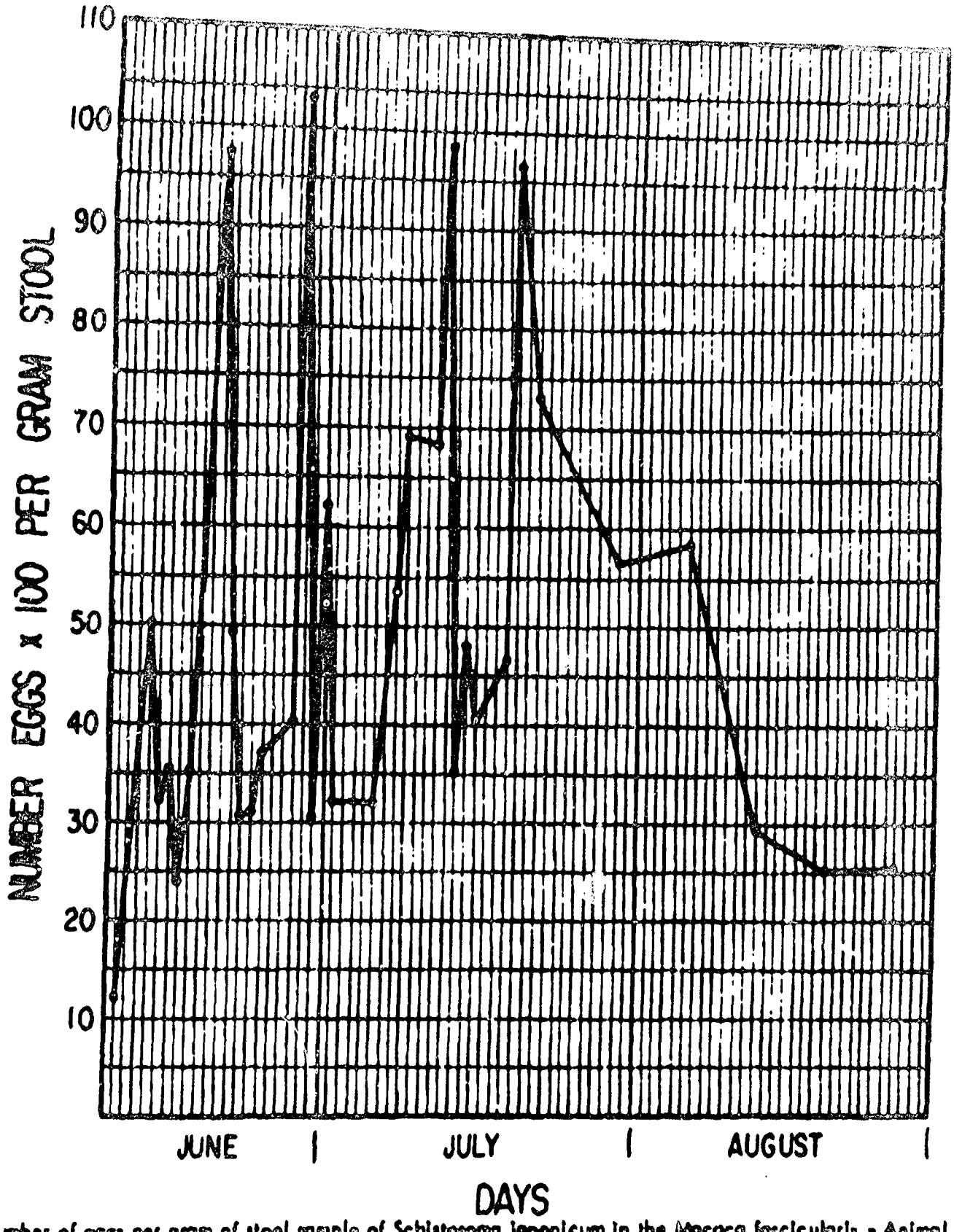
Table II (Continued)

Date	Ova and Parasite and Stool Consistence	# eggs per gram stool
7 June 1976	Formed	328
8 June	Mushy formed	546
9 June	Mushy formed	200
10 June	Formed	208
11 June	Formed	196
14 June	Formed	394
15 June	Formed	342
16 June	Mushy formed	312
17 June	Mushy formed	212
18 June	Mushy formed	186
21 June	Mushy formed	252
22 June	Mushy formed	446
23 June	Formed	174
24 June	Mushy formed	197
25 June	Formed	162
28 June	Mushy formed	322
29 June	Mushy	392
30 June	Mushy	228
1 July	Formed	162
2 July	Mushy formed	194
6 July	Mushy formed	182
7 July	Formed	152
8 July	Mushy formed	332
9 July	Mushy formed	214
12 July	Mushy formed	196
13 July	Mushy	364
14 July	Mushy formed	146
15 July	Mushy formed	92
16 July	Mushy formed	86
19 July	Mushy formed	102
20 July	Mushy formed	64
21 July	Mushy formed	34
22 July	Mushy formed	42
23 July	Formed	52
30 July	Formed	92
6 Aug	Formed	58
13 Aug	Formed bloody	74
20 Aug	Formed	40

Note: 10 Mar 76 petechial hemorrhages occur at the site of infection.
15 Mar 76 petechial disappear.



Number of eggs per gram of stool sample of Schistosoma japonicum in the Macaca fascicularis - Animal A.



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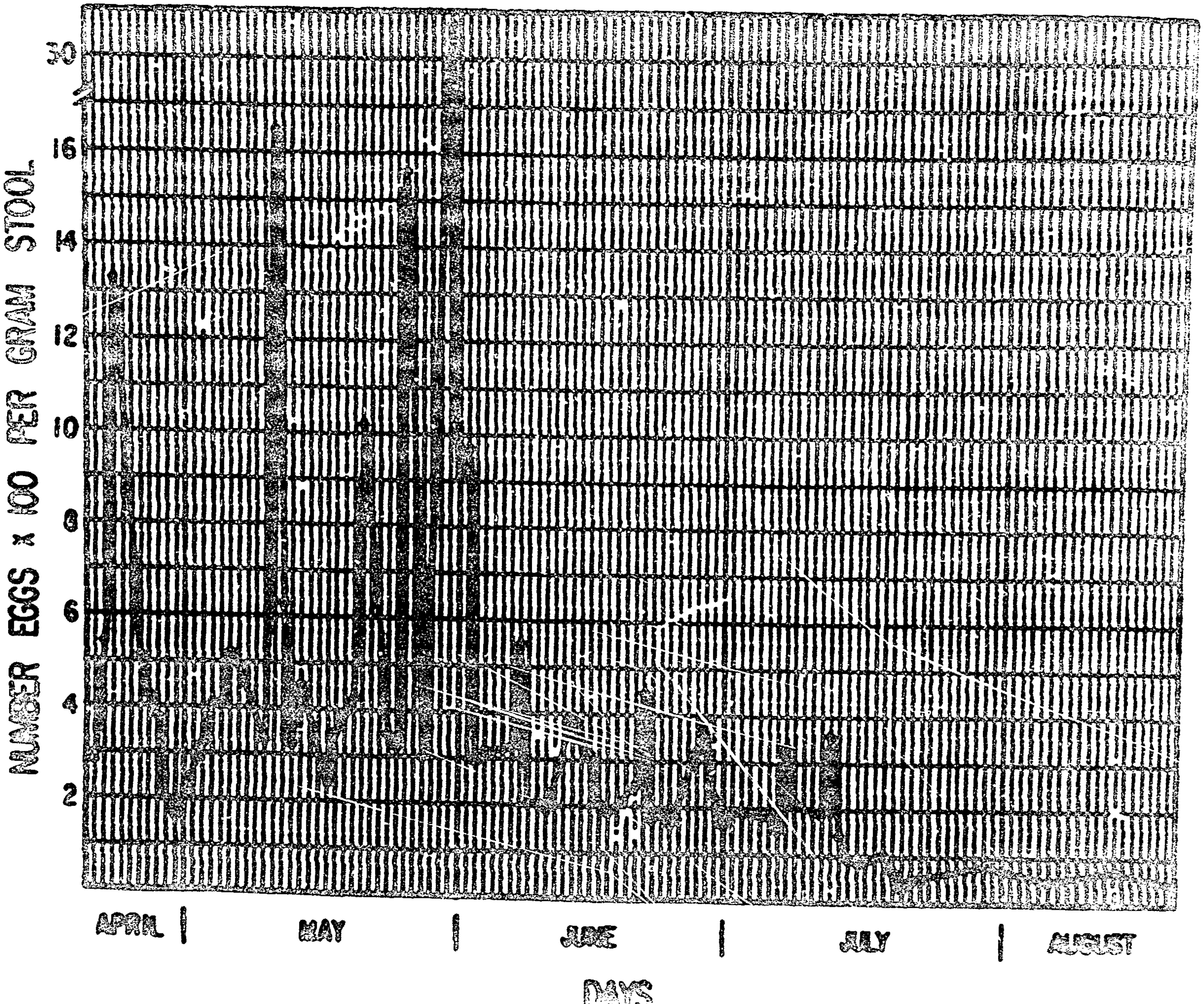

Number of eggs per gram of stool sample of _Schistosoma japonicum_ in the _Macaca fascicularis_ - Animal B.

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